mL. The results were all negative. Each organism (1 x 10^7/mL) was also spiked to a positive Strep A control (3 x 10^5 CFU/mL) to confirm that the test results are the same as expected.

Organism Tested	BioSign® Strep A Test Results	
at 1 x 10^7/mL…	without Strep A	spiked with Strep A
Candida albicans (ATCC 14053)	–	+
Corynebacterium diphtheria (ATCC 296)	–	+
Escherichia coli (ATCC 11775)	–	+
Klebsiella pneumoniae (ATCC 13883)	–	+
Neisseria gonorrhoeae (ATCC 9793)	–	+
Neisseria lactamica (ATCC 23970)	–	+
Neisseria meningitidis serogroup B (ATCC 13090)	–	+
Neisseria sicca (ATCC 9913)	–	+
Proteus vulgaris (ATCC 6059)	–	+
Pseudomonas aeruginosa (ATCC 10145)	–	+
Staphylococcus aureus Cowan (ATCC 12600)	–	+
Staphylococcus epidermidis (ATCC 14990)	–	+
Streptococcus group B (ATCC 12386)	–	+
Streptococcus group C (12388)	–	+
Streptococcus group D (ATCC 27284)	–	+
Streptococcus group F, Type 2 (ATCC 12392)	–	+
Streptococcus group G (ATCC 12394)	–	+
Streptococcus pneumoniae (ATCC 6303)	–	+
Negative Control	–	+
Positive Control	+	+

Reproducibility Study:

Reproducibility of **BioSign® Strep A** test results was examined at two POL (physician's office laboratory) sites and a clinical laboratory, using a total of 15 blind control samples for a total of 90 tests. The panel consisted of 5 negative samples, 5 low positive samples containing approximately 3 x 10^5 CFU/mL, and 5 medium positive samples containing approximately 1.2 x 10^6 CFU/mL, prepared from known live cultures of ATCC strain 19615. The results obtained at each site agreed 100% with expected results.

Distribution of Random Error:

Twenty blind samples prepared by spiking 4 different concentrations of group A streptococcal antigen, prepared from a known live culture of ATCC strain 19615, were separately tested by two operators. Five (5) replicate samples were prepared for each concentration: high positive samples containing approximately 4.8 x 10^6 CFU/mL, medium positive samples containing approximately 1.2 x 10^6 CFU/mL, low positive samples containing approximately 3 x 10^5 CFU/mL, and negative samples. The test results obtained by the two operators showed complete agreement.

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Symbols Key

	Manufactured by
CE	CE Mark
EC REP	Authorized Representative
IVD	*In Vitro* Diagnostic Medical Device
REF	Catalog Number
	Consult Instructions for Use
LOT	Batch Code
EXP YYYY-MM-DD	"Use By" date in year-month-day format
2°C (35°F) Min / 30°C (86°F) Max	Temperature Limitation
Σ n	Contains sufficient for <n> tests
	Do not reuse
CONT	Contents
DEV	Test Device
PIP	Transfer Pipette
TUBE	Extraction Tube
SWAB	Throat Swab
SOL A	Extraction Solution A
SOL B	Extraction Solution B
CONT +	Positive Control
IFU	Instructions for Use
TEST STREP A	Strep A Antigen Detection Test

EC REP
MT Promedt Consulting GmbH
Altenhofstrasse 80
66386 St. Ingbert
Germany
+49-68 94-58 10 20

Manufactured by
PBM
Princeton BioMeditech Corporation
4242 US Hwy 1, Monmouth Jct.
New Jersey 08852 U.S.A.
1-732-274–1000 www.pbmc.com

BioSign® Strep A

Direct Group A Streptococcus Antigen Test

For *In Vitro* Diagnostic Use

Immunoassay for the Detection of Group A Streptoccal Antigen Directly from Throat Swab Specimens

PBM

CLIA Complexity: Moderate
CDC Analyte Identifier Code: 5810
CDC Test System Identifier Code:

Catalog No. BSP-181 25 Test Kit

Intended Use

BioSign® Strep A is a rapid immunochromatographic assay for the detection of group A streptococcal antigen directly from throat swab specimens. The test is intended for use as an aid in the early diagnosis of group A streptococcal infection (1).

Summary and Explanation

Group A streptococcus is one of the most significant human pathogens causing acute pharyngitis, tonsillitis, impetigo, and scarlet fever (1). It is very important to differentiate streptococcal infection from other etiologic agents (e.g., viral, mycoplasmal, or chlamydial) so that appropriate therapy may be initiated. Early diagnosis and treatment of group A streptococcal pharyngitis infections will reduce the severity of symptoms and further complications such as rheumatic fever and glomerulonephritis (2-6). Unlike classical methods for identification, which require 18–48 hours of culture time for throat swab specimens or other exudates to produce results showing bacitracin susceptible beta-hemolytic streptococci, the **BioSign® Strep A** test requires only 7 minutes after collection of the specimen.

Principle

BioSign® Strep A is a rapid immunochromatographic assay for the qualitative detection of group A streptococcal antigen directly from throat swabs. The **BioSign® Strep A** test involves the chemical extraction of group A streptococcal antigen followed by solid-phase immunoassay technology for the detection of extracted antigen. In the test procedure, a throat swab specimen is collected, placed into a mixture of Reagents A and B, and extracted for 2 minutes. The extract is added to the Sample well with the aid of a transfer pipette and is allowed to soak in. If group A streptococci are present in the specimen, they will react with anti-Strep A indicator antibody coupled to dye particles, migrate through the membrane as antigen-antibody-dye complexes, bind to the immobilized anti-Strep A antibody on the membrane, and generate a colored line in the Test window. The rest of the sample and unbound/bound dye complexes continue to migrate to the Control window where antibody to the anti-Strep A indicator antibody is immobilized. At this line, anti-Strep A indicator antibody-unbound/bound dye complexes form a Control line in the Control window. Presence of two colored lines, one in the Test window and the other in the Control window, indicates a positive result, while the absence of a line in the Test window indicates a negative result.

Materials and Reagents

Materials Provided

Each **BioSign® Strep A** test kit contains enough reagents and materials for 25 tests.

- **BioSign** test devices (25): Contain a membrane coated with rabbit anti-group A streptococcus antibody for the test line and a second control antibody, and a conjugate pad impregnated with the rabbit anti-Strep A antibody–dye complex in a protein matrix containing 0.1% sodium azide.
- Extraction Reagent A (6.5 mL): 2.0 M sodium nitrite solution. (Warning: Avoid contact with eyes or skin.)
- Extraction Reagent B (6.5 mL): 0.2 M phosphoric acid solution. (Warning: Irritant. Avoid contact with eyes or skin.)
- Positive Control (1 mL): Extracted (non-infective) group A streptococcus antigen in phosphate buffered saline containing 0.1% sodium azide.
- Extraction Tubes (25)
- Transfer Pipettes (25)
- Throat Swabs (25): Rayon swab with plastic shaft (use only the swabs supplied).
- Instructions for Use

Materials Required but not Provided

- Timer
- Reaction tube rack

Precautions

- For *in vitro* diagnostic use only.
- Do not interchange materials from different lots.
- Do not use kit components after the expiration date.
- The test kit should be used only with the swabs supplied with the kit.
- Do not interchange caps among reagents.
- Use separate, clean transfer pipettes for different specimens.
- Reagent A and B are slightly caustic. Avoid contact with eyes, sensitive mucous membranes, cuts, abrasions, etc. If these reagents come in contact with the skin or eyes, flush with a large volume of water.
- Do not smoke, eat or drink in areas where the specimens or kit reagents are handled.
- Wear disposable gloves while handling the kit reagents or specimens and wash hands thoroughly afterwards.
- All patient samples should be handled as if they are capable of transmitting disease. Observe established precautions against microbiological hazards throughout all procedures and follow standard procedures for proper disposal of specimens.
- The **BioSign® Strep A** device should remain in its sealed pouch until ready for use. Do not use if the pouch is damaged or the seal is broken.
- The control solutions contains sodium azide, which, on contact with lead or copper plumbing, may react to form explosive metal azides. Use a large volume of water to flush reagents on disposal.

Storage and Stability

The **BioSign® Strep A** test kit should be stored at 2–30°C (35–86°F) in its original sealed pouch. Avoid direct sunlight. Do not freeze. Kit contents are stable until the expiration date printed on the outer box.

Specimen Collection and Preparation

Collect throat swab specimens following standard clinical procedures, using the sterile rayon swabs supplied with this kit.

- Swabs should be processed within 4 hours after collection, unless they are stored refrigerated (2–8°C). If stored refrigerated, swabs should be processed within 24 hours from collection.
- If a culture is required, it is recommended that two swab samples be collected. The first swab should be used for testing with **BioSign® Strep A** as soon as possible after collection. The second swab may be stored in a liquid medium (about 200 µL) such as Modified Stuart's or equivalent, for up to 24 hours in a refrigerator.

Procedure

Procedural Notes

The instructions below must be followed carefully to achieve optimal test results. Follow the assay procedure and always perform the test under carefully standardized conditions.

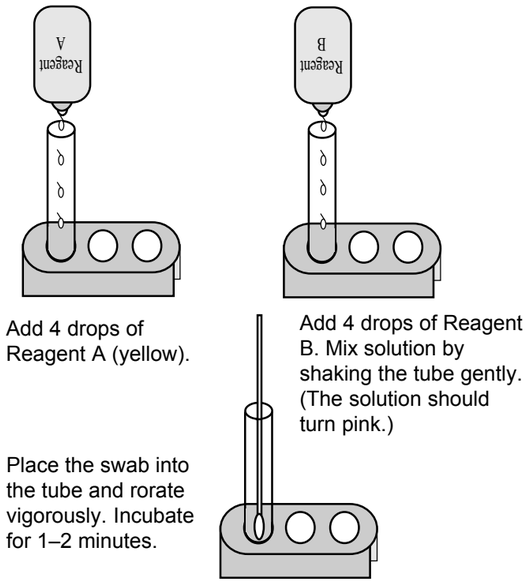
- If specimens, kit reagents or **BioSign** devices have been stored in the refrigerator, allow them to reach room temperature before use.
- Do not open the foil pouch until you are ready to perform the test.
- Several tests may be run at one time.
- To avoid cross contamination, use a new transfer pipette for each specimen.

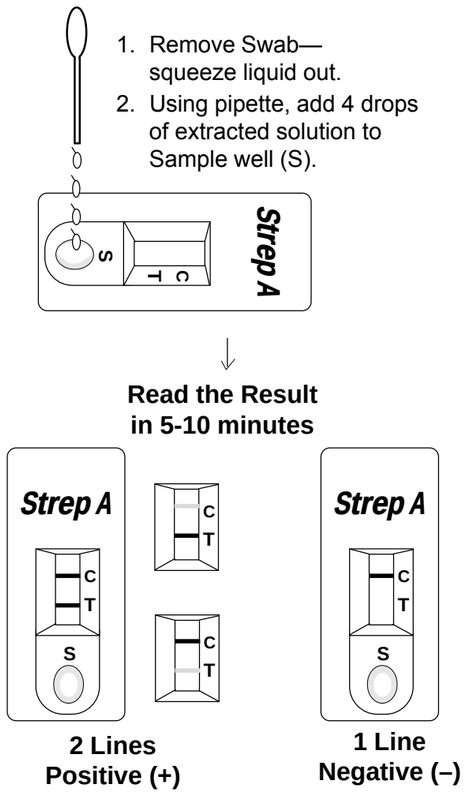
- To avoid contamination of reagents, do not allow the dropper tips of the reagent bottles to come in contact with the extraction tubes.
- Label the device with the patient's name or control number.
- To add Reagents A and B, hold the bottle in a vertical position above the extraction tube and dispense 4 drops into the tube.
- To add extract, allow the transfer pipette to fill with extract and dispense 4 drops of extract into the Sample well, holding the pipette in a vertical position.
- If colored solution migrates through the membrane in the Test window (T), but no Control line forms after 3 minutes, you may not have used enough sample volume. In such a case, you may add an additional 1-2 drops of extracted sample. Insufficient sample volume may cause slow migration and/or incompletion of the assay (invalid test result).
- After testing, dispose of the **BioSign** device, throat swab, extraction tube and transfer pipette following proper laboratory practices. Consider any material that comes into contact with specimen to be potentially infectious.

Test Protocol

1. Dispense 4 drops of Reagent A (yellow) into extraction tube.
2. Add 4 drops of Reagent B into the extraction tube. Mix solution by shaking the tube gently. (The solution will turn pink.)
3. Immediately place the specimen swab in the extraction tube. Rotate the swab vigorously in the extraction solution to extract specimen thoroughly.
4. Let stand for 1–2 minutes.
5. Remove the swab—squeeze the liquid out of the swab. Discard the swab.
6. Add 4 drops (90-120 µL) of the extracted solution into the Sample well (S) using a transfer pipette.
7. Read the result in 5 minutes, after a distinct color line has formed in the Control window (C), but no later than 10 minutes after the extracted solution has been added to the Sample well.

TEST PROCEDURE





Interpretation of Results

Positive: Two colored lines, one at the Test position (**T**) and the other at the Control position (**C**), indicate that group A streptococcal antigen has been detected.

*Note: The test result can be read as soon as a distinct purplish-red line appears at the Test position (**T**) and at the Control position (C). The Test line may appear before the Control line (strong positive case) or after the Control line (weak positive case), and the Test line may be darker or lighter than the Control line. Any visible Test line indicates a positive result.*

Negative: Only one colored line at the Control position (**C**), and no distinct colored line at the Test position (**T**), indicates that group A streptococci have not been detected. A clear background in the Result window is considered an internal negative procedural control. This result indicates that the specimen is a presumptive negative for the presence of group A streptococcal antigen. It is recommended by the American Academy of Pediatrics (7) that presumptive negative results be confirmed by culture.

Invalid: A distinct colored line in the Control position (**C**) should always appear. The test is invalid if no line forms at the Control position in 5 minutes.

Limitations

- As is the case with any other diagnostic procedure, the results obtained with this kit must be used only as an adjunct to other information available to the physician.
- This test should be used only for the qualitative detection of strep A antigen. Use of the kit for the semi-quantitative determination of group A strep has not been established.
- This test will not differentiate between a carrier and an infected individual.

- The **BioSign® Strep A** test can detect non-viable as well as viable organisms. The test may therefore detect organisms which cannot be demonstrated in culture.
- This test is not intended as a substitute for bacterial culture testing; test results should be compared with culture identification until each laboratory establishes its own equivalences of performance. Additional follow-up testing using the culture method is recommended if the **BioSign® Strep A** test result is negative and group A streptococcal infection is suspected.
- Test specimens heavily colonized with *Staphylococcus aureus* (> 10^{10} CFU/mL) can yield false positive results.
- Proper throat swabs must be obtained for good quality tests.
- Pharyngitis can be caused by organisms other than group A streptococcus. This test does not provide any further information about pharyngitis other than the possibility of strep A infection. If clinical signs and symptoms are not consistent with laboratory results, a follow-up throat culture and grouping procedure should be performed. Pharyngitis is also caused by other serological groups of streptoccoccus as well as other organisms.
- A negative result may be obtained due to poor sample collection, or at the onset of the disease due to a low antigen level, below the sensitivity limit of the test. If symptoms persist or intensify, repeat testing with a fresh sample is recommended. Test the fresh sample by culture method to confirm the negative test result obtained with **BioSign® Strep A** (7).
- Swabs transported in liquid media prior to testing may result in reduced sensitivity due to dilution of organisms.

User Quality Control

External Quality Control:

- Good laboratory practice recommends the use of external positive and negative controls to assure the test reagents are working properly and that the user has performed test correctly. If the controls do not perform as expected, review the instructions for use to see if the test was performed correctly and repeat the test or contact PBM Technical Assistance before performing patient specimens. The built-in purplish-red Control line indicates only the integrity of the test strip and proper fluid flow.
- It is recommended that the control test be performed, using the controls provided, before using a new lot or shipment of **BioSign® Strep A** kits to confirm the expected Q.C. results. The frequency of additional Q.C. tests should be determined according to your laboratory's standard Q.C. procedures. Upon confirmation of the expected results, the kit is ready for use with patient specimens.
- The Positive control will produce a moderate positive result (two lines) when the test has been performed correctly and the test strip is functioning properly. Add 4 drops each of Reagents A and B into an extraction tube, then add one drop of Positive Control and mix thoroughly. Process the extraction in the same manner as you would for a patient specimen according to the **Test Procedure**.
- The Negative control will yield a negative result (Control line only) when the test has been performed correctly and the test device is functioning properly. Add 4 drops each of Reagents A and B into an extraction tube, then add one drop of Negative Control and mix thoroughly. Process the extraction in the same manner as you would for a patient specimen according to the **Test Procedure**.
- In addition to the external positive control provided with the kit, a known live culture of *Streptococcus pyogenes* (strep A) such as ATCC strain 19615 can be used for quality control testing. Live culture from an agar plate may be collected by swab and tested the same way as described for unknown samples in the **Test Procedure**. Negative control can be used to dilute the culture organism to make a Positive control.
- A known live culture of group C streptococci such as ATCC strain 12388 can be used for negative quality control testing at a minimum concentration of 10^6 inactivated CFU per mL. Process the extraction in the same manner as you would for a patient specimen according to the **Test Procedure**.
- The Positive and Negative controls provided with the kit do not monitor the extraction step. If the controls do not perform as expected, do not report patient results.
- The use of positive and negative controls from other commercial kits has not been established with **BioSign® Strep A**.

Internal Procedural Control

- A colored line in the Control line area can be considered an internal positive procedural control. A distinct pinkish-purple control line will always appear if the test has been performed correctly. If the control line does not appear, the test is invalid and a new test should be performed. If the problem persists, contact PBM for technical assistance.
- A clear background in the result area is considered an internal negative procedural control. If the test is performed correctly and the test strip is working properly, the background in the result area should be clear, providing a distinct negative result.

Expected Results

Group A streptococcus infection exhibits a seasonal variation and is most prevalent in the winter and early spring. Approximately 19% of all upper respiratory tract infections are caused by group A streptococcus (7). The highest incidence of this disease is found in high density populations, such as school aged children and military bases. Males and females are equally affected by the disease (8).

Performance Characteristics

Clinical Correlation:

The performance of **BioSign® Strep A** was compared to that of conventional plate culture techniques in a prospective evaluation of clinical specimens. Throat swab specimens were collected from 505 child and adult patients with pharyngitis symptoms. Each swab was first used to inoculate a sheep blood agar plate containing a bacitracin disk, and the swab was then assayed with **BioSign® Strep A**. The plates were incubated at 37°C in 5% CO_2 for 18-24 hours to detect ß-hemolytic colonies typical of group A streptococci. If the plates were negative, they were held for an additional 18-24 hours. All samples were collected from cultured plates and assayed after 18-24 or 36-48 hours by a Strep A confirmatory latex agglutination test (Streptex by Murex). All presumptive positive ß-hemolytic colonies were serotyped by four other kinds of Streptex test kits (B, C, F and G). Serotyping by five kinds of Streptex test kits (A, B, C, F and G) was also performed when borderline ß-hemolytic results were obtained, or when a negative ß-hemolytic colony was observed. These results constitute the confirmed 18/48 hour culture results. The results are summarized below.

		BioSign® Strep A (+)	(–)	**Total**
Confirmed 18/48 Hour Culture Results	(+)	127	5	**132**
	(–)	5	368	**373**
Total		**132**	**373**	**505**

Relative Sensitivity ($^{127}/_{132}$): **96.2%**
Relative Specificity ($^{368}/_{373}$): **98.7%**
Overall Accuracy ($^{495}/_{505}$): **98.0%**

Clinical Assay Sensitivity:

The minimum detection limit of the test is 1.5 x 10^5 CFU/test. This was established by testing a known number of organisms, ATCC 14285 or ATCC 19615, using Todd Hewitte Broth from BBL. The cultured organisms were serially diluted in culture medium and tested by **BioSign® Strep A**. The same dilutions were cultured overnight on sheep blood agar plates from BBL for cell enumeration in CFU/mL. The assay results are as follows:

Cell Number (CFU/mL)	**BioSign® Strep A Results**
6.0 x 10^5	++ (medium positive)
3.0 x 10^5	+ (low positive)
1.5 x 10^5	+ (low positive)
7.7 x 10^4	– (negative)
3.8 x 10^4	– (negative)

Clinical Assay Specificity:

To confirm the specificity of **BioSign® Strep A**, organisms likely to be found in the respiratory tract, as listed below, were tested at 1 x 10^7 organisms per